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β-D-1,3 GLUCANASES IN FUNGI[1]

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Abstract

β-D-1,3 Glucanases are of common occurrence in fungi, being detected in the culture filtrates of 96% of the organisms tested in shake flasks and in the sporophores of six basidiomycetes. The enzyme is constitutive. Basidiomycete QM 806 and *Sporotrichum pruinosum* QM 826 are excellent sources of β-D-1,3 glucanase of the exo-type giving glucose as the sole reducing product of laminarin hydrolysis. *Rhizopus arrhizus* QM 1032 produces a β-D-1,3 glucanase of the endo-type giving laminaribiose and higher oligosaccharides as the products of hydrolysis of β-D-1,3 glucans. By controlling the conditions of growth, β-D-1,3 glucanases can be produced free of β-1,4 glucanase (cellulase).

Introduction

β-D-1,3 Glucanases are enzymes hydrolyzing the β-D-1,3-linked polymers of glucose (glucans). Previously they have been known as laminarinases because they were originally tested on laminarin, a polysaccharide from the marine alga, *Laminaria*. Since β-D-1,3 glucans are now known to be of wide occurrence (algae, fungi, higher plants) it seems desirable to give a general name to the enzymes attacking them. A historical review of the nature and occurrence of the glucans is found in the book by Whistler and Smart (16).

The function of β-D-1,3 glucans in plants is not clear. Even less seems to be known of how they are formed or stored. They act as a reserve food in *Laminaria*, and perhaps, also, in sclerotia of the fungus *Poria cocos* (15). In other organisms, they appear to be related to structural materials. The glucan is part of the cell wall in baker's yeast (3). It is found as callose in grape vines (2). In both of the latter, the glucan exists in a somewhat modified form. β-D-1,3-Linked glucose units occur also in polysaccharides of mixed linkage types.

β-D-1,3 Glucanases are ubiquitous enzymes, having been found in fungi (1, 13), bacteria (4), higher plants (16), algae (5), lower forms of sea animals (snails, giant chiton (7)). The greater ease of detection of the enzyme as compared with the substrate perhaps accounts for the wider variety of sources reported for the glucanase. Most reports on the β-D-1,3 glucanases have been incidental to some other problem. Recently (11) a more direct interest has been shown.

In the present report we focus attention on this particular group of enzymes. How frequently do they occur in fungi? How do they hydrolyze their substrata? Can they be obtained free of other polysaccharases? These are some of the questions we hope to answer in part.

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Methods

The organisms were grown on a carbon source in a basal medium containing (per 1000 ml): KH_2PO_4, 2.0 g; $(NH_4)_2SO_4$, 1.4 g; urea, 0.3 g; $MgSO_4.7H_2O$, 0.3 g; $CaCl_2$, 0.3 g; yeast extract, 0.1 g; and trace elements (Fe, 1.0 μg; Mn, 0.5 μg; Co, 0.5 μg; Zn, 0.8 μg). The cultures (50 ml/250-ml flask) were grown on a reciprocal shaker (90 strokes/minute) at 29° C.

The enzyme-containing solutions were obtained by filtering the cultures through medium porosity, fritted-glass crucibles. Some of the enzyme solutions were concentrated and partially purified by solvent precipitation. Other solutions were dialyzed, concentrated by evaporation (Rotovap), and lyophilized.

The laminarin used in these tests is insoluble laminarin, Batch I.L.31 from the Institute of Seaweed Research, Inveresk, Scotland. It has a moisture content of 11%. The glucose yield on acid and on enzyme hydrolysis indicates that 83% of the dry weight is glucan. The cellulose (Solka Floc) is a highly purified wood product of Brown Co., Berlin, New Hampshire.

β-D-1,3 Glucanase activity was determined as follows: 0.6% laminarin in *M*/20 citrate pH 4.8, 0.5 ml; enzyme solution to be assayed, 0.5 ml; in Folin tubes, 40° C 1 hour.

Reducing sugars produced were determined by the dinitrosalicylic acid method of Sumner and Somers (14). An enzyme solution has one β-D-1,3 glucanase unit (U) per ml if, when assayed as shown, it produces 0.5 mg of reducing sugar as glucose. Cellulase (Cx) was determined by hydrolysis of carboxymethylcellulose as previously described (10).

Paper chromatograms were developed for 16 to 20 hours with isopropanol: acetic acid : water (67:10:23). Reducing sugars were detected with benzidine (6). The movement of compounds is expressed as a comparison with the distance moved by glucose, i.e., R_G = 1.00 for glucose.

Enzyme names and abbreviations used herein include:

β-D-1,3 glucanase = laminarinase = polysaccharase acting on long-chain β-D-1,3-linked glucose polymers;

β-D-1,3 oligase = β-D-1,3 glucosidase = β-D-1,3 oligosaccharase acting on short-chain β-D-1,3-linked glucose polymers. This is a somewhat arbitrary separation of enzyme types and may require modification.

Cx = β-1,4 glucanase = cellulase (in part) = polysaccharase acting on long-chain β-1,4-linked glucose polymers.

Results

A. Screening of Fungi for β-D-1,3 Glucanase

One hundred and forty organisms were grown in shake flasks and tested for β-D-1,3 glucanase. All but five produced detectable amounts of the enzyme. The most active cultures are listed in Table I. Penicillia, especially of the *P. luteum* series, appear frequently in this list, but the 12 aspergilli tested produced lower amounts of the enzyme.

TABLE I

Fungi producing high yields of β-D-1,3 glucanase

Organism	QM No.	Carbon source*	β-D-1,3 glucanase, U/ml	Cx = β-1,4 glucanase, U/ml
Alternaria dauci	7170	Cellobiose	7	0
Basidiomycete sp., conidial	806	Cellobiose	160	1.5
Basidiomycete sp., conidial	806	Starch	900	0.2
Basidiomycete sp., conidial	807	Cellobiose	160	1.6
Basidiomycete sp., conidial	592	Cellobiose	10	0.9
Basidiomycete sp., conidial	594	Cellobiose	50	1.3
Basidiomycete sp., conidial	2378	Cellobiose	60	3.2
Humicola fuscoatra	34*e*	Inulin	9	NT†
Myrothecium verrucaria	460	Laminarin	10	0
Paecilomyces varioti	10*a*	Laminarin	8	0
Penicillium echinulo-nalgiovense	7301	Cellulose	13	15.0
Penicillium funiculosum	474	Cellobiose	8	0
Penicillium javanicum	1876	Laminarin	15	0
Penicillium javanicum	6959	Cellulose	24	13.0
Penicillium nigricans-janczewskii (series)	6900	Cellulose	7	16.0
Penicillium pusillum	137*g*	Cellobiose	11	(+)
Penicillium rotundum	1854	Laminarin	24	0
Penicillium spiculisporum	979	Laminarin	38	0
Penicillium stipitatum	6759	Laminarin	10	0
Penicillium vermiculatum	1858	Laminarin	28	0
Penicillium vermiculatum	7316	Cellulose	33	8.0
Penicillium wortmanni	1859	Laminarin	14	0
Pestalotiopsis westerdijkii	381	Cellulose	9	35.0
Poria cocos	7695	Laminarin	40	0.1
Poria monticola	1010	Laminarin	23	0.5
Rhizopus arrhizus	1032	Cellobiose	24	0
Sporotrichum pruinosum	826	Cellulose	600	72.0
Stemphylium sp.	7086	Cellobiose	9	0
Syncephalastrum racemosum	709	Laminarin	18	0
Trichoderma viride	6*a*	Maltose	6	0

* Carbon source 0.5%; shake flasks.
† NT = no test.

Seven of the fungi were grown on 10 carbon sources (Table II). β-D-1,3 Glucanase was produced in cultures on all substances that supported growth. Yields were no better on laminarin than on other substrates. The enzyme is therefore constitutive in fungi. In this respect, it differs from cellulase and chitinase, which are adaptive (10).

The fruiting bodies of six basidiomycetes were collected in the field and tested for the presence of β-D-1,3 glucanase and other polysaccharases (Table III). β-D-1,3 Glucanase was the dominant polysaccharase in four of these species, one of which, *Polyporus betulinus*, also contained amylase as a major enzyme. Amylase was the dominant polysaccharase present in the sporophore of *Lactarius piperatus*; and cellulase (Cx) in *Clavaria* sp. These two fungi contained little or no β-D-1,3 glucanase. None of the fungi tested had detectable amounts of dextranase (α-D-1,6 glucanase) or polygalacturonase (α-D-1,4 polygalacturonase). Chitinase was present only in trace amounts (18-hour assay). The enzyme values given are based on extracts of fresh sporophore tissue. The amount of β-D-1,3 glucanase is very low compared

TABLE II

Effect of carbon source (0.5%) on β-D-1,3 glucanase production by fungi

Organism	QM No.	β-D-1,3 glucanase U/ml when grown on: Glycerol	Mannitol	Glucose	Xylose	Lactose	Maltose	Cellobiose	Laminarin	Starch	Cellulose
1. *Asp. phoenicis*	1005	0.5	2	5	6	NG†	5	5	5	6	NT†
2. Basidiomycete	806	0.1	2	464	222	NG	395	282	264	416	60
3. *Myrothecium verrucaria*	460	4	1	4	5	0.4	6	7	11	8	2
4. *Paciliomyces variati*	10a	3	4	4	3	NG	3	4	4	4	NG
5. *Rhizopus arrhizus*	1032	1	1	0.8	0.9	NG	1	1.8	1	0.9	NG
6. *Sporotrichum pruinosum**	826	47	13	14	21	NG	15	33	15	19	76
7. *Trichoderma viride*	6a	1	1	2	2	4	8	1	4	10	8

* For *S. pruinosum* 0.1% protease peptone was included.
† NT = no test; NG = no growth.

TABLE III

Polysaccharases found in sporophores of basidiomycetes

Fungus	Sporophores Type	% moisture	Enzyme per gram fresh weight: β-D-1,3 glucanase, U/g	Amylase, α-1,4 glucanase, U/g	β-1,4 xylanase, U/g	Cx, β-1,4 glucanase, U/g
Lepiota procera	Mushroom	86	14.0	0.4	0.6	0.0
Calvatia cyathiforme	Puffball	NT	9.0	0.2	0.3	0.0
Tremella foliacea	Gelatinous	95	1.0	0.0	0.0	0.0
Polyporus betulinus	Bracket	82	9.0	1.5	0.1	0.3
Lactarius piperatus	Mushroom	NT	0.3	6.0	0.0	0.0
Clavaria sp.	Coral	90	0.6	0.0	0.6	16.0

with the amounts produced by fungi growing in shake flasks (Table I). An "average" fungus in shake culture produces about 100 times as much enzyme per unit weight.

Of the commercial enzyme preparations we have examined, only the following have appreciable β-D-1,3 glucanase activity: cellulase (Takamine), 3.1 U/mg; hemicellulase (Nutr. Biochem. Co.), 1.2 U/mg; enzyme 19AP (Röhm and Haas), 0.9 U/mg; and lipase (Gen. Biochem. Co.), 0.4 U/mg. Traces of activity only were found in mylase P (NBC), pectinol 10 M (R and H), diastase, malt (GBC), ficin (Merck), pectinase (NBC), and takadiastase (Parke Davis Co.). No activity could be detected in: pancreatin (NBC), steapsin (NBC), glucose oxidase (Takamine), lactase (NBC), trypsin (Eimer and Amend), invertase (NBC), pepsin (Merck). These data indicate that commercial sources contain relatively little β-D-1,3 glucanase (compare with Table IV).

Three of the active fungi (Table II) were selected for further study: Basidiomycete QM 806, *Sporotrichum pruinosum* QM 826, and *Rhizopus arrhizus* QM 1032.

1. Basidiomycete QM 806

This fungus is the best producer of β-D-1,3 glucanase that we have found. It grows well on a variety of carbon sources (Table II), and (fortunately) can produce β-D-1,3 glucanase in the absence of cellulase (β-1,4 glucanase). Starch (1%), used for the routine production of the enzyme, is rapidly digested (4–7 days), during which time the pH falls from 6.2 to 3.0 and then rises again to 6.5. The β-D-1,3 glucanase levels increase rapidly after the starch has been consumed, reach a maximum at 10–14 days, and then decrease.

The highest yields on starch have been 900 β-D-1,3 glucanase units per ml of culture medium. In these solutions the Cx is barely perceptible (0.1–0.2 U/ml). On the other hand, when grown on cellulose, this organism produces high yields of cellulase (about 100 Cx U/ml), with relatively low yields of β-D-1,3 glucanase (40–60 U/ml).

Basidiomycete QM 806 has been submitted to taxonomists who specialize in identification of basidiomycetes in culture. Unfortunately, it is an unfamiliar

species and remains unidentified. It produces conidia in culture and has clamp connections. It has been suggested that we place it in the form genus *Ptychogaster*. Our QM culture collection has several similar isolates, apparently the same fungus, all originating in tropical areas. All are cellulolytic, produce very good yields of β-D-1,3 glucanase, and behave alike in culture. Known species of basidiomycetes have been tested for comparison, but produced low levels of β-D-1,3 glucanase: *Ptychogaster rubescens* (QM 1011), *Schizophyllum commune* (QM 812), *Coprinus sclerotigenus* (QM 933), and *Polyporus versicolor* (QM 1013). *Poria cocos* (QM 7695, QM 7696) and *Poria monticola* (QM 1010) produced yields of enzyme only a little lower than those obtained from some members of the Basidiomycete 806 complex (see Table I).

2. Sporotrichum pruinosum QM 826

This organism was selected for study because of the high β-D-1,3 glucanase yields (approaching those of QM 806), and because it simultaneously produces very high yields of cellulase (Cx) when grown on cellulose. When grown on other substrates it still produces good β-D-1,3 glucanase yields, but little or no cellulase.

Cellulose is the best of the tested carbon sources for β-D-1,3 glucanase (and Cx) production by *Sporotrichum pruinosum* QM 826 (Table II). Cellulase develops earlier than β-D-1,3 glucanase (Fig. 1A). In this respect β-D-1,3 glucanase resembles amylase in its development in cellulolytic cultures (10). The yields of enzyme are a function of the substrate concentration (Fig. 1B), highest yields being obtained at the highest concentration tested (2%). Unfortunately, the incubation time to reach maximum values also increases.

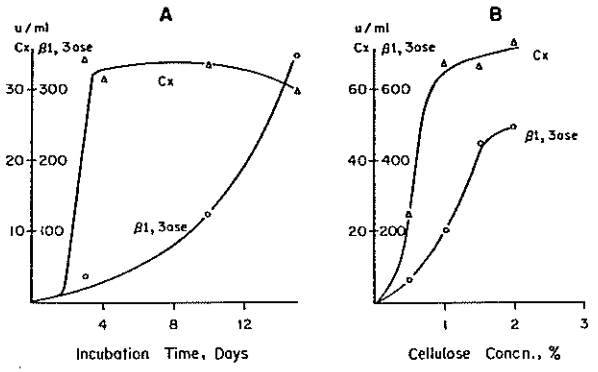

FIG. 1. Production of β-D-1,3 glucanase, and of Cx, by *Sporotrichum pruinosum* QM 826. A. Development of activity during incubation. Substrate = 0.5% Solka Floc (cellulose) + 0.1% protease peptone. B. Effect of cellulose concentration on yields. Medium includes 0.1% protease peptone. Experiment lasted 32 days.
△ — △ Cx, ○——○ β-D-1,3 glucanase.

For a 2-week incubation period, 1.5% cellulose is optimal. Addition of protease peptone (0.1%) increases the cellulase (Cx) while decreasing the β-D-1,3 glucanase yields of this organism.

3. Rhizopus arrhizus QM 1032

This organism produces much less β-D-1,3 glucanase than do Basidiomycete QM 806 and *Sporotrichum pruinosum*. Its interest lies in the difference between its glucanase and that of the other two fungi, and in the relative absence of a β-D-1,3 oligase, features which make it possible to accumulate intermediates in the hydrolysis of β-D-1,3 glucans.

The low yields (about 1 U/ml) obtained on the basal medium (Table II) were improved markedly by the addition of 0.1% protease peptone. On 0.6% cellobiose yields of 24 U/ml were obtained (vs. 1.0 U/ml on cellobiose in absence of peptone). (In our assay all reducing sugar produced is determined as glucose. Since *Rhizopus arrhizus* produces mostly dimer and trimer, the unit value is not strictly comparable to the unit obtained with organisms producing largely glucose.) The yields on glucose and on glycerol were about half those on cellobiose. No cellulase was found in any of these filtrates.

B. Concentration and Purification of β-D-1,3 Glucanase

The enzymes of the culture solutions were precipitated by the addition of two volumes of cold acetone. The precipitates were fractionated to some extent by dissolving and reprecipitating with increasing concentrations of ethyl alcohol. In *Sporotrichum pruinosum*, most of the β-D-1,3 glucanase (80%) was soluble in 50% alcohol, but insoluble in 75% alcohol. Some of the enzyme preparations are listed in Table IV, with data also for cellulase activities.

TABLE IV

A comparison of the best enzyme preparations

Source of enzyme	Grown on:	β-D-1,3 glucanase, U/mg	Cx, U/mg
Basidiomycete QM 806	Starch	352.0	0.2
Basidiomycete QM 806	Cellulose	17.0	137.0
Sporotrichum pruinosum QM 826	Cellulose	370.0	37.0
Rhizopus arrhizus QM 1032	Cellobiose	40.0	0.0

C. Properties of the β-D-1,3 Glucanases of Basidiomycete QM 806, Sporotrichum pruinosum QM 826, Rhizopus arrhizus QM 1032

1. Enzyme Activity

pH.—The pH optimum for β-D-1,3 glucanase of all three fungi is about 4.5 in citrate buffer. There is very little activity above pH 7.0 or below pH 3.0. The *Rhizopus* enzyme is inactive below pH 4.0.

Temperature.—Under the conditions of the assay (pH 4.8, 1 hour, laminarin 3 mg/ml), maximum activity takes place at 60° C (Fig. 2B, for QM 806).

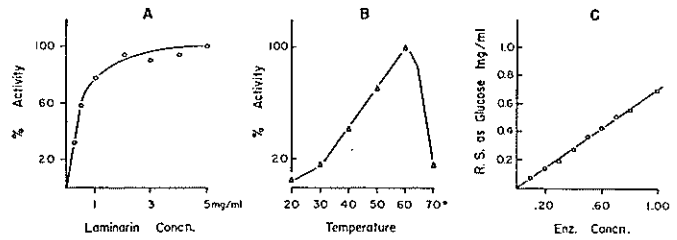

FIG. 2. β-D-1,3 Glucanase of Basidiomycete QM 806. A. Effect of substrate concentration, in reaction mixture (time, 30 minutes). B. Effect of temperature (pH, 4.7; time, 60 minutes). C. Effect of enzyme concentration (time, 30 minutes).

Inactivation is rapid at 70° C. The *Rhizopus* enzyme is slightly less heat stable than the others. At pH levels other than the optimum, inactivation is much more rapid. At pH 6.5, for instance, 90% of the enzyme is inactivated in 30 minutes at 50° C.

Substrate concentration.—The maximum rate of activity is reached at substrate concentrations of 2 mg/ml of reaction mixture (Fig. 2A). Below this value, the rate falls off sharply.

Enzyme concentration, and time of incubation.—Under the conditions of the assay, straight line relationships hold for sugar production vs. enzyme concentration (Fig. 2C), and for sugar production vs. time of incubation. Deviation from this occurs when the amount of reducing sugar (as glucose) exceeds 0.90 mg/ml.

Substrate specificity.—Our β-D-1,3 glucanase preparations are contaminated with other enzymes, and as a result we have not yet thoroughly investigated substrate specificity. We have tried pachyman (from sclerotia of *Poria cocos*) and found that the enzyme hydrolysis products (glucose, laminaribiose, laminaritriose) correspond with those obtained from laminarin. This supports the earlier identification of pachyman as a predominantly β-D-1,3-linked glucan (15).

2. Hydrolysis Products of Laminarin-Using Enzymes

Laminarin has been hydrolyzed by our β-D-1,3 glucanase preparations (Table V).

The low value for *R. arrhizus* is due to the fact that the reaction was not continued to the time of glucose appearance. The values for hydrolysis by Basidiomycete QM 806 and by *Sporotrichum pruinosum* enzymes agree with the value for acid hydrolysis.

Chromatograms of the hydrolyzates of laminarin (Fig. 3) indicate that glucose is produced directly by the action of β-D-1,3 glucanases of Basidiomycete QM 806, and of *Sporotrichum* (latter not shown). At no time during the hydrolysis is there more than a trace of intermediate (at most, a trace of

TABLE V

Enzyme hydrolysis products of laminarin

Preparation from:	Extent of hydrolysis*	Products
Basidiomycete QM 806	83%	Glucose
Sporotrichum pruinosum QM 826	83%	Glucose
Rhizopus arrhizus QM 1032	43%	Dimer; trimer

* From reducing value calculated as glucose.

dimer). In contrast to this is the hydrolysis by *Rhizopus*. Here dimer (R_G 0.69) and trimer (R_G 0.33) are produced quickly, but glucose appears only after long incubation.

The immediate production of glucose from laminarin by the preparation from Basidiomycete QM 806 might be due to the presence of an oligase acting on intermediates. To test this we measured oligase activity using as substrate the dimer–trimer mixture produced by *Rhizopus* enzyme acting on laminarin. As a measure of oligase activity we used the time required to produce 1 mg of glucose (determined with glucose oxidase). There is very low oligase activity compared with the amount of β-D-1,3 glucanase activity in preparations of any of these organisms (Table VI). There is enough, however, that on long hydrolysis and with strong enzyme solutions both intermediates are hydrolyzed to glucose (Fig. 3 shows this for *Rhizopus*).

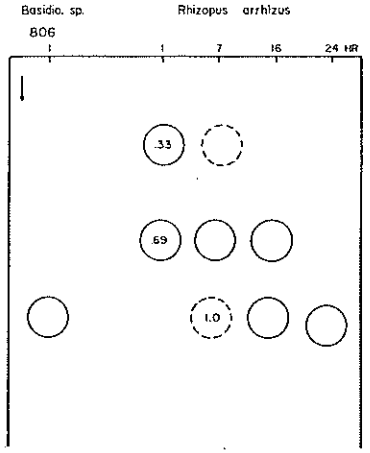

FIG. 3. Chromatograms of enzyme hydrolyzates of laminarin. 806 = β-D-1,3 glucanase of Basidiomycete QM 806. Numbers in spots = R_G values, i.e., the distance moved relative to that moved by glucose. Broken circles indicate fainter spots.

TABLE VI

Time required to produce 1 mg of glucose per ml by 1 unit of β-D-1,3 glucanase

Enzyme of:	Time, hours*	
	From laminarin	From dimer–trimer
Basidiomycete QM 806	2.5	240
Sporotrichum pruinosum QM 826	2.0	68
Rhizopus arrhizus QM 1032	70.0	72

* Reaction mixture: substrate 3 mg/ml, pH 4.8, 40° C, 1 unit β-glucanase.

The trimer disappears much more rapidly than the dimer. The basidiomycete filtrate produces glucose 100 times as fast from laminarin as from an equal concentration of dimer–trimer mixture; the *Sporotrichum* filtrate, 30 times as fast. Thus, the absence of dimer and trimer in hydrolyzates of laminarin by these filtrates is not due to an active oligase, but must be due to direct production of glucose from laminarin. On the other hand, the *Rhizopus* filtrate produces glucose at about the same rate from laminarin and from dimer–trimer mixture, the hydrolysis of the latter being the limiting factor.

The diverse nature of the glucanase systems of Basidiomycete QM 806 and of *Rhizopus* was brought out in another type of experiment (Fig. 4). The glucanase of Basidiomycete QM 806 was added to the *Rhizopus*–laminarin hydrolyzates at various times. In similar enzyme systems, a doubling of enzyme concentration usually gives an increased over-all reaction. But here the action of the glucanase of Basidiomycete QM 806 was markedly inhibited

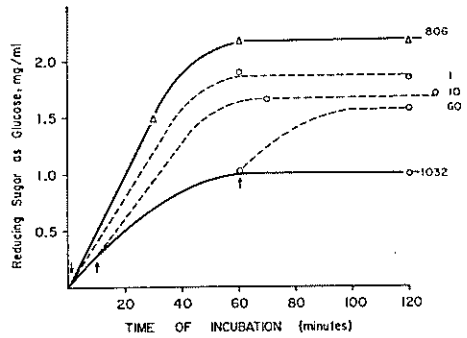

FIG. 4. Inhibition of activity of one β-D-1,3 glucanase (806) by the prior action of another (1032) on laminarin. 806 = glucanase of QM 806 alone; 1032 = glucanase of QM 1032 alone; 1, 10, 60 = glucanase of QM 1032 acting alone for 1, 10, 60 minutes, at which time (↑) the glucanase of QM 806 was added. These hydrolyzates have a full complement of both enzymes.

by prior action of the *Rhizopus* glucanase for as little time as 1 minute. Apparently, the action of the *Rhizopus* enzyme produces short chains which are resistant to attack by the Basidiomycete QM 806 enzyme.

Discussion

A. Occurrence and Production of β-D-1,3 Glucanase

β-D-1,3 Glucanases are present in most fungi. They are secreted into the medium where they function as digestive enzymes hydrolyzing glucans produced by other organisms. Extracellular digestive enzymes (cellulase, chitinase, xylanase) are usually adaptive in fungi. The β-D-1,3 glucanases are constitutive. They may also function in another capacity, one common to all fungi, the intracellular hydrolysis (and synthesis) of a reserve material containing β-D-1,3 glucosidic linkages. As such, these enzymes resemble amylase which is also constitutive in many fungi, which may function intra- and extra-cellularly, and which appears later in the growth cycle than cellulase and other adaptive enzymes. Glycogen, a substrate for amylase, is well known as a reserve food in fungi, but β-D-1,3 glucans have been reported in only three fungi: baker's yeast, *Poria cocos*, and perhaps *Sclerotinia libertiana* (8). *Poria cocos* is the only fungus we have tested which is known to produce both the substrate β-D-1,3 glucan and the enzyme β-D-1,3 glucanase. We expect that further investigations will show that β-D-1,3 glucans are of rather widespread occurrence in fungi.

Although nearly all fungi produce β-D-1,3 glucanase, there are great differences in the amounts produced by the various organisms. *Myrothecium verrucaria* and *Aspergillus niger*, organisms which have been used as sources of β-D-1,3 glucanase in the works of others, (1, 13) are not very good producers of this enzyme. Likewise, most commercially available enzyme preparations contain little of this polysaccharase. Yet β-D-1,3 glucanase is about as common in fungal enzyme preparations as is amylase. This contamination of enzyme preparations should be considered in reporting on the specificity of enzymes for any particular linkage, e.g., the ability of cellulase to hydrolyze polysaccharides containing linkages other than β-1,4 (9, 12).

The organisms which we have studied are excellent sources of β-D-1,3 glucanase. The yields obtained are higher than those from other known sources by a factor of 10–100. It is interesting that the production of a constitutive enzyme should be influenced greatly by the carbon source, and that the carbon source best for each organism should be different. Thus, growth on soluble starch leads to the greatest production of β-D-1,3 glucanase in Basidiomycete QM 806 whereas cellulose gives the lowest yields. On the other hand, cellulose is the best carbon source for *Sporotrichum pruinosum*, and cellobiose seems to be a superior carbon source for the production of the enzyme by *Rhizopus arrhizus*. Growth conditions may be affecting the build-up of a reserve β-D-1,3 glucan, and this in turn may determine the level of enzyme produced.

B. Mode of Action

β-D-1,3 Glucanases are of two types (11) which we might designate (after Duncan *et al.* (5)):

(*a*) The endo- or random-splitting type. Hydrolysis of β-D-1,3 glucans by this enzyme yields laminaribiose and higher oligosaccharides. It is found in wheat, barley, rye, marine algae, and in *Rhizopus arrhizus*.

(*b*) The exo- or endwise-splitting type. Hydrolysis by this enzyme produces glucose as the initial and sole product. Found in almond emulsin and in fungi (Basidiomycete QM 806, *Sporotrichum pruinosum*, etc.).

This classification is based on the products of hydrolysis, but like all such schemes, it may go beyond the facts. The action of the exo-type enzymes implies the removal of a single glucose from chain A, followed by removal of a unit from chain B, etc., leading eventually to what we find to be a rather resistant dimer. Assuming a degree of polymerization of 20 for laminarin, the dimer would amount to about 10% of the product and should be readily detectable by our techniques. Its apparent absence suggests the possibility of an "unzippering" action whereby each molecule of laminarin is completely hydrolyzed to glucose before the enzyme moves on to the next molecule of substrate. More data are required to resolve this problem.

Investigations by others indicate that the β-D-1,3 glucanases may be a family of enzymes, each fungus producing one or more members of the family. In *Aspergillus niger* three β-D-1,3 components were found and these differed from the three β-1,4 glucanases (= cellulases (13)). Other workers have investigated the possibility that phosphorolysis might be involved but only negative results (5) were reported.

What part, if any, does a β-D-1,3 oligase (glucosidase) play in the hydrolysis of a β-glucan? By analogy with other polysaccharide-splitting systems, there should be a role for an oligase. Certainly the endo-β-D-1,3 glucanase should be complemented by an enzyme whose function it is to hydrolyze the dimer and trimer. For the exo-β-D-1,3 glucanase systems, there seems to be no requirement for an oligase, glucose being the immediate product of the reaction.

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References

1. Aitkin, R. A., Eddy, B. P., Ingram, M., and Weurman, C. Action of culture filtrates of fungus *Myrothecium verrucaria* on β glucosans. Biochem. J. **64**, 63–70 (1956).
2. Aspinall, G. O. and Kessler, G. Callose from the grape vine. Chem. & Ind. London, 1957, 1296 (1957).
3. Bell, D. J. and Northcote, D. H. The structure of a cell-wall polysaccharide of baker's yeast. J. Chem. Soc. 1950, 1944–1947 (1950).
4. Chesters, C. G. C., Turner, M., and Apinis, A. Decomposition of laminarin by microorganisms. 2nd Int'n Seaweed Symposium, Trondheim, 1955, 141–144 (1956).

5. Duncan, W. A. M., Manners, D. J., and Ross, A. G. Enzyme systems in marine algae. Carbohydrase activities. Biochem. J. **63**, 44–51 (1956).
6. Horrocks, R. H. Paper partition chromatography of reducing sugars with benzidine as a spraying reagent. Nature, **164**, 444 (1949).
7. Huang, H. and Giese, A. C. Digestion of algal polysaccharides by marine herbivores. Science, **127**, 475 (1958).
8. Kitahara, M. and Takeuchi, Y. Chemical components of sclerotia of *Sclerotinia libertiana*. Gifu Daigaku Nōgakubu Kenkyū Hōkoku, **8**, 100–105 (1957) (seen only in abstract).
9. Kooiman, P. Some properties of cellulase of *Myrothecium verrucaria* and some other fungi. Enzymologia, **18**, 371–384 (1957).
10. Mandels, M. and Reese, E. T. Induction of cellulase in *Trichoderma viride*. J. Bacteriol. **73**, 269–278 (1957).
11. Manners, D. J. Enzymic degradation of polysaccharides. Quart. Revs. London, **9**, 73–99 (1955).
12. Ploetz, T. and Pogacar, P. Specificity of β glucanases. Ann. **562**, 36–44 (1949).
13. Stone, B. A. Complexity of β glucanases from *Asp. niger*. Biochem. J. **66**, 1p–2p (1957).
14. Sumner, J. R. and Somers, G. F. Laboratory experiments in biological chemistry. Academic Press, Inc., New York. 1944.
15. Warsi, S. A. and Whelan, W. J. Structure of pachyman, the polysaccharide component of *Poria cocos* Wolf. Chem. & Ind. London, 1957, 1573 (1957).
16. Whistler, R. L. and Smart, C. L. Polysaccharide chemistry. Academic Press, Inc., New York. 1953.